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13. ABSTRACT (Maximum 200 words)

The initial synthetic work was started at April of 2003. The primary objectives were to synthesize hybrid sugar and aminoglycoside antibiotic with N-1 modification. The synthesis of pyranmycin with hybrid sugar has been accomplished. The synthetic approach of N-1 modification has yielded several aminoglycosides that are active against various resistant bacteria. In addition, we have devoted effort for the development of kanamycin class aminoglycosides. Several compounds with low micromolar activity against resistant bacteria have been synthesized.

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II. Scientific Progress and Accomplishments

Highlights:

1. Novel Pyranmycin class aminoglycosides that are against various resistant bacteria have been synthesized.
2. Novel kanamycin class aminoglycosides with activity against various resistant bacteria have been synthesized.
3. New methodology for the purification of small-scale of aminoglycoside has been developed.

Details:

1. Progress on Hybrid Sugars Approach

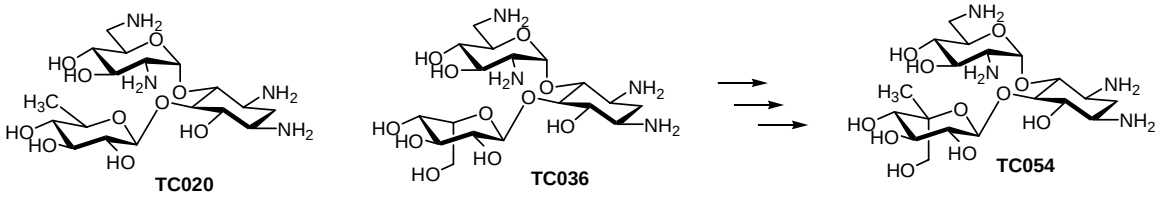
We have completed the synthesis of the proposed pyranmycin with hybrid sugar, **TC054**, which is earlier than the proposed timeline. In addition, we have also synthesized two new pyranmycin families, **TC036** and **TC037**, as the comparison for our hybrid sugar design (Scheme 1). **TC036** contains a L-idopyranose as the ring III component, while **TC037** has a 6-deoxy- L-idopyranose. Glycosylation followed by the standard deprotection protocol, **TC036** and **TC037** were obtained. The antibacterial result confirms our speculation that having the 6-hydroxyl group or 6-amino group of the ring III L-pyranose is essential for the antibacterial activity of pyranmycin. On the other hand, having a methyl group as the ring III D-pyranose will increase the activity. However, when both structural features are combined in the same pyranose as in the case of **TC054**, the expected addition effect was not observed. In fact, these two structural features work against each other resulting in a dramatic decrease of the antibacterial activity.

In light of this finding, we have learned valuable information summarized as follow:

A. The traditional strategy that many pharmaceutical companies are employing for drug development involves identifying the effective structural modules (pharmacophores) and combining these modules to the same lead(s). Our result has proved that such approach may not be an effective method. Synthetic methodologies that can provide compounds with more structural aspects for identifying leads are essential.

B. In the area of aminoglycoside antibiotics, more effort is needed for studying how aminoglycoside antibiotics are imported into bacteria.

Scheme 1. Design of hybrid sugar approach

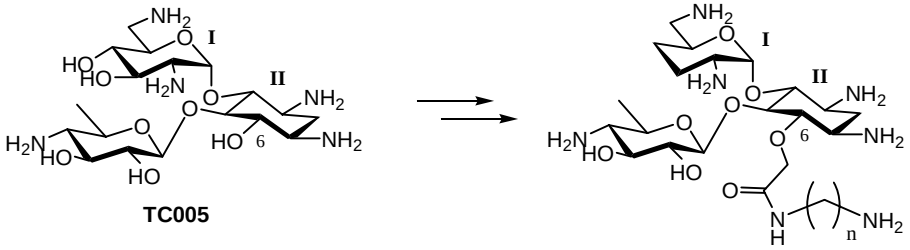


With the setback of the unexpected low activity of pyranmycin with the hybrid sugar project (**TC054**), we decide to initiate the O-6 modification approach, a new approach that is designed to increase the antibacterial activity of the synthesized aminoglycosides.

2. Progress on O-6 Modification: Changed from Hybrid Sugars Approach

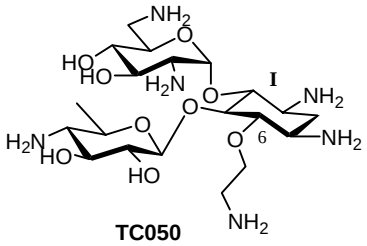
The original design is to use **TC005** as the lead and introduce a side chain at the O-6 position. The attachment of linear side chain has been shown to increase the antibacterial activity of neamine (rings I and II) (Scheme 2).

Scheme 2. Synthesis of designed pyranmycin with O-6 modifications



Initially, the synthesis has encountered great difficulties. Although we have completed the synthesis of one of the designed compound, **TC050**, the lengthy synthesis and relatively low yield prevent us from the scale-up production or further derivatization (Figure 1). Therefore, an alternative designs were proposed. In the new designs, we wish to incorporate two new structural features together and separately. The two features are 3',4'-dideoxggenation and N-1 modification. The latter will be discussed in the N-1 modification section. Both structural features are known to enable the synthetic aminoglycosides become active against aminoglycoside resistant bacteria.

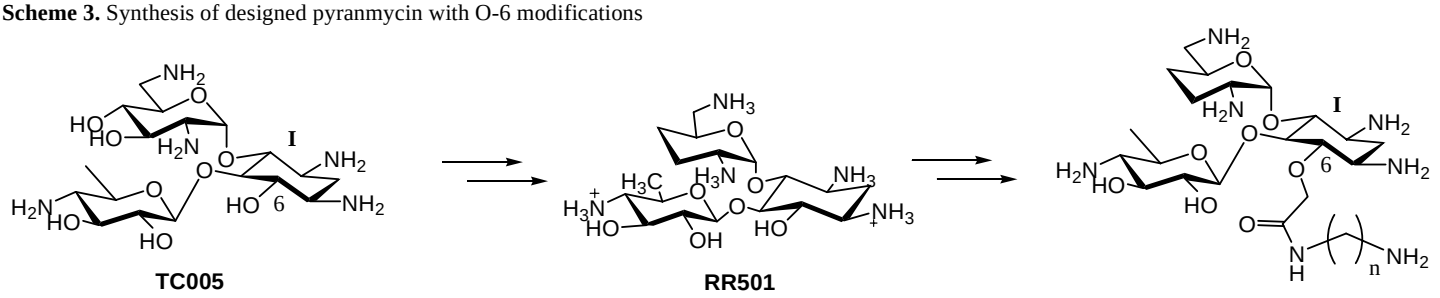
Figure 1.



MIC = 10 and 0.8 μM against *E. coli* and *S. aureus*, respectively

The modified strategy for 3',4'-dideoxggenation is shown in Scheme 3. The compound designed to demonstrate the activity against resistant bacteria, **RR501** has been synthesized. As expected, **RR501** was found to be active against certain aminoglycoside resistant bacteria. The continuing work for attaching side chain at O-6 position has been carried out.

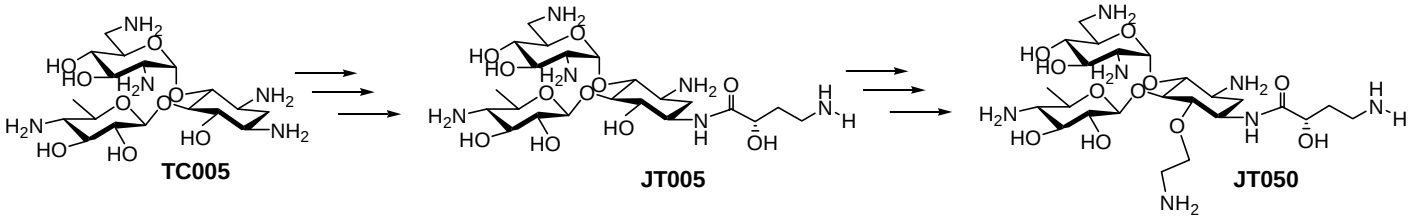
Scheme 3. Synthesis of designed pyranmycin with O-6 modifications



3. Progress on N-1 Modification Approach

Aminoglycosides with attachment of (S) 4-amino-2-hydroxybutanoyl (AHB) group at N-1 position has long been known to be one of the most effective methods for developing new aminoglycoside against resistant bacteria, which has led to the development of semi-synthetic amikacin. Our strategy in this objective is to introduce AHB at the N-1 position of the lead pyranmycin, **TC005**. It is expected that the designed compound, **JT005**, will be active against several clinically significant resistant strains of bacteria (Scheme 4). As mentioned previously, we also intend to introduce side chain at the O-6 position such as in the design of **JT050**.

Scheme 4.

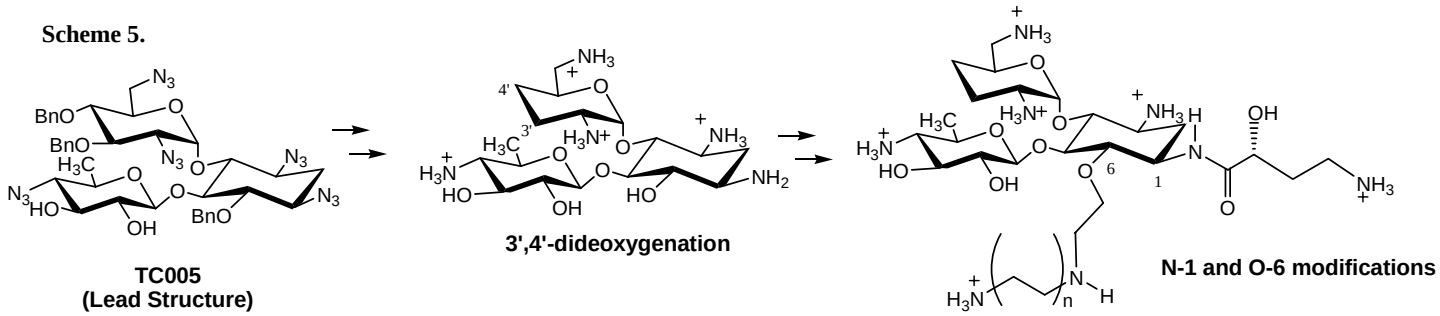


Initially, the synthesis of **JT005** has encountered great challenges. For example, we have spent more than six months in developing and optimizing the condition for the selective modification of N-1 amino group alone. Nevertheless, the ideal condition and method have been developed, and **JT005** has been synthesized. However, an unexpected obstacle occurred. The product was mixed impurities that were difficult to separate. We were hoping to obtain extra support for the purchase of HPLC system that can be used for the purification of **JT501**.

As we were exploring the possibility of purchasing a HPLC system from Dionex for the purification purpose, a modified synthetic route has been developed with the aim of providing purer compound. The modified route was developed successfully. In addition, we finally developed a protocol for the purification of synthesized aminoglycosides after extensive trials. The protocol can be applied to both pyranmycin and kanamycin classes including those with various structural modifications. It can be employed to the sample as little as 10 mg. After such a purification process, the antibacterial activities of most of the synthesized aminoglycosides were improved.

Currently, our effort is focusing on the synthesis of pyranmycin with a combination of 3',4'-dideoxygenation and N-1 modification as illustrated in Scheme 5.

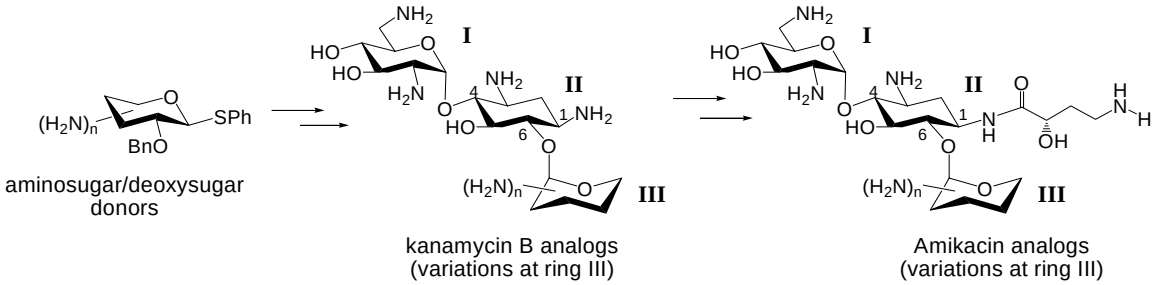
Scheme 5.



4. Progress on Kanamycin B Analogs

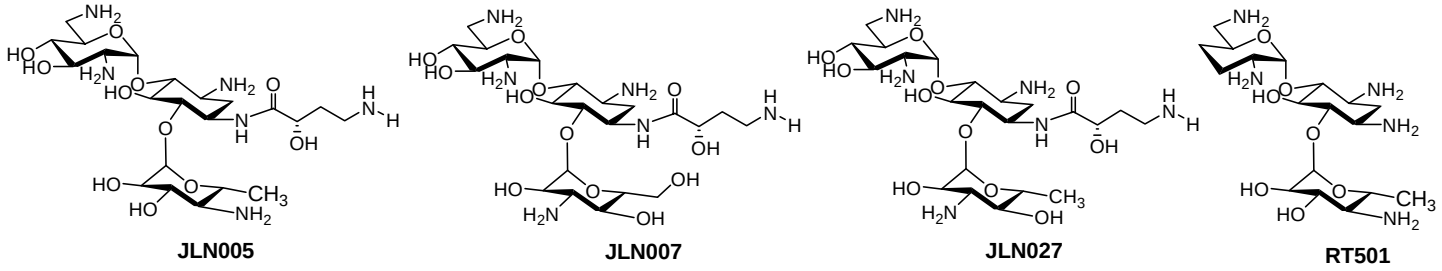
This is the newly developed project that is not in the original proposal. However, we have discovered that we can move into this project based on what we have learned from our previously work without too much additional cost. The overall strategy is to synthesize a library of kanamycin B analogs that will be active against resistant bacteria as outlined in Scheme 6.

Scheme 6. Synthesis of kanamycin and Amikacin Analogs



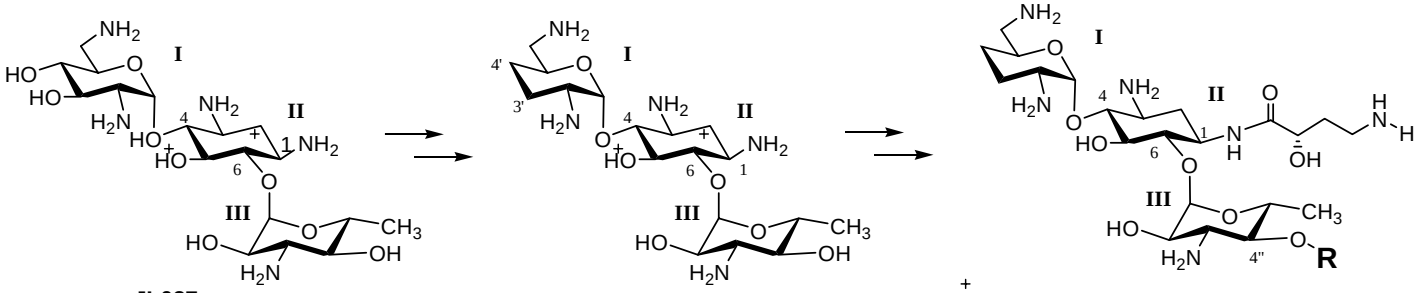
We have completed the synthesis of more than twenty kanamycin B analogs. From the preliminary SAR, we have identified the O-4" position of ring III as the optimal position for further modifications. We also plan to incorporate the structural features of 3',4'-dideoxggenation and N-1 modification. Several compounds, **RT501**, **JLN005**, **JLN007**, and **JLN027** with these features were prepared and assayed (Figure 2). As expected, these compounds posses modest to excellent activity (low micromolar) against aminoglycoside resistant bacteria.

Figure 2.



Similar to the pyranmycin project, our current effort is focusing on the synthesis of kanamycin with a combination of 3',4'-dideoxygenation, N-1 modification, and O-4" modification as illustrated in Scheme 7.

Scheme 7.



5. Antiviral activity of aminoglycoside antibiotics

The screening of pyranmycins against two types of RNA molecules in HIV-1 virus has been completed (Table 1). **TC009** appears to be the best candidate, having the highest inhibition against the functions of both Rev and Tat while processing reasonably low cell toxicity. More **TC009** has been sent to the Southern Research Institutes for further studies. However, due to the limited personal involved in my project, I will focus more on the development of antibacterial agents. Nevertheless, I'll keep evaluating the potential designs of antiviral agents, and synthesize these agents when appropriate.

Table 1. Anti-Rev and Anti-Tat Activities of Pyranmycins

Entry	Compound	Test Concentration	HIV-1 Rev Assay		HIV-1 Tat Assay	
			% Inhibition of Rev Function[a]	% Reduction in Cell Viability[b]	% Inhibition of Tat Function[a]	% Reduction in Cell Viability[b]
1	**TC001**	50 μM	0.0	0.0	10.3	0.0
2	**TC002**	50 μM	0.0	0.0	36.6	0.0
3	**TC003**	50 μM	0.0	0.0	20.4	0.0
4	**TC004**	50 μM	0.0	0.0	15.2	0.0
5	**TC005**	50 μM	0.0	0.0	46.5	0.0
6	**TC006**	50 μM	0.0	0.0	17.1	0.0
7	**TC007**	50 μM	8.9	0.0	28.7	0.0
8	**TC009**	50 μM	49.6	12.3	14.1	0.0
9	**TC010**	50 μM	15.8	10.9	42.4	4.6
10	**TC012**	50 μM	21.9	16.1	4.9	11.6
11	**TC015**	50 μM	3.0	6.8	25.4	9.9
12	**TC016**	50 μM	16.6	13.2	71.1	13.2
13	**TC017**	50 μM	24.3	11.6	49.9	11.0
14	**TC018**	50 μM	38.4	18.4	70.4	13.6
15	**TC019**	50 μM	38.1	11.6	46.3	5.4
16	**TC021**	50 μM	14.7	0.0	65.2	0.0
17	**TC022**	50 μM	0.0	6.0	7.7	0.0
18	**TC038**	100 μM	29.4	22.5	20.8	21.0
19	**TC040**	100 μM	30.9	20.5	12.7	11.6
20	**TC041**	100 μM	21.9	14.4	20.4	21.4
21	**TC044**	100 μM	28.6	1.8	50.9	20.6
22	**TC045**	100 μM	100.0	99.9	100.0	100.0
23	**Leptomycin B**[c]	100 nM	100.0	60.6	98.3	73.8
24	**Ro24-7429**[d]	1 μM	53.4	35.5	100.0	42.8

[a] Renilla Luciferase analysis, [b] Firefly Luciferase analysis, [c] Reported inhibitor of Rev Function, [d] Reported inhibitor of Tat Function

III. Future Tasks

1. Prepare more aminoglycosides that are active against resistant bacteria for assay of other pathogens, such as anthrax and tuberculosis.
2. Continue the development of more active aminoglycosides.
3. Modify the synthesis to ensure the scale-up production will be feasible for future animal or clinical trials.
4. Identify collaborators for the evaluation of maximum cytoplasmic concentration (Cmax).